COMPARATIVE STUDY ON THE EXTRACTION OF β-CAROTENE IN THE FLESH AND PEEL OF PAPAYA

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ABSTRACT

The objective of this study was to compare and optimize the extraction conditions for β-carotene compounds from flesh and peel of papaya (*Carica* papaya) using extraction process and HPLC analysis also to determine mineral content and microbe affected. Extraction is a process to obtaining something from mixture or compound by chemical or physical methods. Liquid-liquid extraction is used to extract and purify carotenoids from flesh and peel of Papaya and then this sample will be analysis in High Performance Liquid Chromatographer (HPLC).Several parameter was applied to determine the effects of acetone as a solvent, acetone ratio/ concentration (%), extraction temperature (°C), and extraction time (minutes) on β-carotene content from flesh and peel of papaya (*Carica* papaya). The independent variables were coded at five levels and their actual values were selected based on the results of single factor experiments. Results showed that acetone concentration was the most significant factor affecting the concentration of β-carotene. The optimum extraction conditions were found to be acetone concentration of 60%, extraction temperature of 40°C, and extraction time of 60 minutes. Under the optimized conditions, the experimental value for β-carotene content was ($3.45x10^{-5}$ mg/L) for peel and ($3.01x10^{-5}$) mg/L for flesh of papaya

ABSTRAK

Tujuan kajian ini adalah untuk membandingkan dan menentukan nilai kandungan molekul β-carotene dalam keadaan yang optimum dari isi dan kulit daripada buah betik dengan menggunakan proses pengekstrakan dan menganalisis menggunakan alat HPLC dan juga menentukan nilai kandungan mineral. Dari aspek bioteknologi, kajian ini juga mengenalpasti kesan dan tindakbalas hidupan organisma merbahaya seperti bakteria dapat hidup atau sebaliknya. Pengekstrakan ini adalah satu proses penyerapan molekul-molekul tertentu berdasarkan sifat kimia dan fizikal sesuatu molekul dalam larutan kimia.kajian ini adalah salah satu cara mengekstrak molekul β-carotene dan secara langsung dapat menentukan nilai kepekatan molekul β-carotene antara isi dan kulit buah betik. Beberapa parameter telah digunakan dalam kajian ini bagi menentukan kesan optimum terhadap jenis larutan yg digunakan, nisbah larutan dan sample, kesan terhadap masa dan kesan terhadap suhu. Pembolehubah ini direkodkan pada 5 tahap dan nilai ini dipilih berdasarkan daripada setiap keputusan kajian dan ini mendapati larutan acetone memberikan nilai paling terbaik iaitu 60% pada bersuhu 40°C dan pada masa 60 minit. Dibawah keadaan optimum ini, nilai kepekatan molekul β-carotene pada kulit dan isi 3.45×10^{-5} mg/L dan 3.01×10^{-5} mg/L masing-masing

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A	—	Absorbance
ε	—	Molar absorptivity
λ	—	Wavelength
c	—	Concentration
∫	—	Length through the sample

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cultivated papaya, *Carica papaya* L., sometimes known as paw paw (or papaw), is a fast-growing tree-like herbaceous plant in the family Caricaceae. In Australia, red and pink fleshed cultivars are often known as 'papaya' to distinguish them from the yellow-fleshed fruits, known as 'paw paw', but both of these common names refer to the same plant species. Irrespective of its flesh colour, *C. papaya* is generally known as 'papaya' in other countries. In some areas, an unrelated plant, *Asiminia triloba* (Annonaceae), native to north America, is also called paw paw.

Until recently, the Caricaceae was thought to comprise 31 species in three genera (namely Carica, Jacaratia and Jarilla) from tropical America and one genus, Cylicomorpha, from equatorial Africa (Nakasone & Paull 1998). However, a recent taxonomic revision proposed that some species formerly assigned to Carica were more appropriately classified in the genus Vasconcella (Badillo 2002). Accordingly, the family's classification has been revised to comprise Cylicomorpha and five South and Central American genera (Carica, Jacaratia, Jarilla, Horovitzia and Vasconcella) (Badillo 1971), with Carica papaya the only species within the genus Carica (Badillo 2002).

Although opinions differ on the origin of *C. papaya* in tropical America (see Garrett 1995), it is likely that *C. papaya* originates from the lowlands of eastern Central America, from Mexico to Panama (Nakasone & Paull 1998). Its seeds were

distributed to the Carribean and south-east Asia during Spanish exploration in the 16th Century, from where it spread rapidly to India, the Pacific and Africa (Villegas 1997).

Figure 1 β-carotene in Papaya

β-carotene is the compound responsible for orange colour in papaya, carrot and other fruits, and it is also used as a colour ingredient in many food formulations. A great interest has recently been focused on β-carotene due to its preventive activity against several pathologies, such as cardiovascular disease, hepatic fibrogenesis, solar light induced erythema, human papillomavirus persistence and some cancer types, such as prostate, gastrointestinal and epithelial. β-carotene has also been recently reported to play a role in lung function as well as in foetal growth. Finally, it is also important to consider the synergic action of carotenoids with other bioactive compounds present in fruits and vegetables.

Carotenoid analysis in food products may be carried out by different methods: HPLC, or colour evaluation. Although spectrophotometry or colorimetry can be used to rapidly assess the β-carotene content of products derived from papayas, a highly versatile, sensitive and selective method such as HPLC is needed for reliable analysis of food samples. HPLC analysis of carotenoids is usually done with C18 or C30 RP-columns, operated with isocratic or gradient elution with a wide variety of mixtures of different organic solvents as mobile phases, using UV–vis (450 nm) or photodiode array or MS detection. Heating the column is sometimes

used to improve pigment separation as well as to standardize the separation conditions. For the extraction of carotenoids from the samples, different systems can be used, like liquid–liquid extraction, solid phase extraction or supercritical fluid extraction.

AOAC (1993) recommends methanol/tetrahydrofuran (THF) (50:50 v/v) for extracting the carotenoids, while other sources use ethyl acetate (100%) or different mixtures of ethanol/hexane, acetone/ethanol/hexane, ethyl acetate/hexane or acetone/hexane.

The instability of β-carotene during processes of extraction, handling, and elimination of organic solvents makes the preparation of a sample for analysis an extremely delicate task, often requiring successive and complex procedures to ensure that all the carotenoids are extracted. Besides, not all the analytical methods available for carotenoid analysis in food products are suitable for β-carotene rich foods due to the low solubility of β-carotene in some of the solvents employed as in the case of methanol and due to the fact that the use of other solvents may interfere with the mobile phases applied for carotenoid separation. There are many HPLC methods that can be applied to the determination of carotenoids.

However, this kind of compounds needs a very careful and tedious manipulation due to their chemical lability. Therefore the development of new methodologies of extraction/separation is of relevance and the necessity for a reliable and rapid analysis method for β-carotene in vegetable products has been recognized. This work is aimed at solving the problems above mentioned with a quite simple preparation of the samples, the selection of extraction solvent mixtures more compatible with mobile phase, and short run times, by developing a suitable, reliable, rapid and simple HPLC method for β-carotene analysis and its compare it with a reference spectrophotometric method.

West Macedonia and Epirus regions located in Northwestern Greece have a mild and rainy climate in spring and autumn, providing nearly ideal conditions for fungal growth, with temperatures ranging between 8 and 25 1C.Driven by the growing interest in natural, tropical and ethnic foods, the market in Greece for wild edible papaya is expanding, offering larger varieties of papaya to consumers. In addition, papaya consumption has increased during recent years, due to their delicate flavor and texture as well as their high content of trace minerals. Therefore, it is necessary to investigate the metal content in wild species, given the fact that many of them are known to accumulate high levels of heavy metals, such as cadmium, mercury, lead and copper (Kalaˇ c and Svoboda, 2000). These elements are known to have severe toxicological effects on human health even at very low concentrations. Several factors may affect the accumulation and concentration of trace elements and heavy metals in papaya. Concentrations of the elements are generally assumed to the species-dependent, but substrate composition is also considered to be an important factor (Kalaˇ c and Svoboda, 2000; Cocchi et al., 2006).

Preliminary FoodNet data on the incidence of food borne illness show Salmonella at the top of the overall incidence in the United States (Vugia et al., 2002). Reflecting a worldwide trend in the United States, the proportion of those Salmonella isolates that were Salmonella Enteritidis (SE) increased from 6% in 1980 to 25% in 1995 (Altekruse, Cohen, & Swerdlow, 1997). The risk of acquiring a food borne disease has increased greatly. Pathogen survival depends on many factors, including the physical and chemical characteristics of the fruit or vegetable, the post harvest processes applied and consumer handling practices (FDA/ CFSAN, 1999).

Watermelon, melon and papaya are highly popular fruits in Brazil. These fruits are low acid with an average pH above 4.5, and often served sliced in food establishments in fresh pieces in mixes for salad bars, at deli counters and as a pulp juice. Salmonella spp. can survive and grow in these fruits as described by Escartin, Ayala, and Lozano, 1989; Golden, Rhodehamel, and Kautter, 1993; Leverentz et al., 2001; Ukuku and Sapers, 2001 and Viswanathan and Kaur, 2001.

1.2 Objectives of This Study

- To extract β-carotene in the flesh and peel of papaya

1.3 Scopes of This Study

- To study the effect of solvents type in the extraction process to the sample.
- To determine the most optimum parameters that produces high yield of β-carotene in the extraction process
- To investigate mineral content in the flesh and peel of papaya

1.4 Problem Statement

- Privies researchers more focus on flesh and seed of papaya
- Lack study on comparison of mineral content in papaya in other local fruit
- The peel has potential to be converted into added value product
- The demand on β-carotene has increased significantly, with increased of consumer awareness about cancer.

In early this century, scientist found that some cancers can be avoided by β-carotene. The major sources of β-carotene came from papaya. But β-carotene also can be finding in other orange fruits such as orange and carrot. Peel and flesh of papaya containing amount of β-carotene because it is orange in color.

Every single day, we can hear many peoples dies cause of cancers. The increasing of human awareness makes the demand on β-carotene increase significantly with it. So, many companies are trying to get more sources to get this antioxidant. Nowadays, Malaysia is among the larger country that produced the β-carotene product.

The skin of papaya is excellent for treating skin wounds and places that do not heal quickly. All the parts of the papaya fruit are useful and beneficial. Right from the seeds to the papaya leaves and the flesh of the fruit, all of it has some value. Both the inside and the outside of the fruit can be utilized .Thus no part of the fruit is useless or goes as a waste.

CHAPTER 2

LITERATURE REVIEW

2.1 Origin and Distribution

The name for papaya is *Carica papaya* L. It is a tree-like herbaceous plant, a member of the small family Caricaceae and widely cultivated for its edible fruits it was believed in 16th century the papaya seeds were brought to Melaka from Philippines. Papaya is called as “betik “in Malaysia. Papaya is rich in enzyme called as papin (help in tenderizing meat), vitamin C (more than orange that is 71 mg verses 39.6 mg) and a lot of fibres. You can easily see the Papaya tree everywhere in Malaysia. Papaya, native to Central America and Mexico but it is now a very common fruit grown in most of the tropical and subtropical countries. Some countries called it 'pawpaw' but do not be confused with another unrelated species of fruit that goes by this actual name too.

Papaya is green when young and will turn yellowish-orange when ripe while its flesh is yellow, orange or red, depending on the various cultivars. Some varieties can grow to an enormous size (above 4kg/10 lbs), especially those from the South America origins. There are numerous small black seeds clustered in the center. It is edible and tastes spicy, similar to black pepper but it is usually not well appreciated. The papaya tree is an unbranch tree and can grow till 10 m tall. The 7 lobes leaves are large and place on top of the trunk. It’s about 45cm to 65cm. The flower is small and a bit waxy. We can see them on the axils of the leaves. The lower trunk is filled with the old scars and you can see them when the tree grows higher and higher. These scars were made by the old leaves or the borne fruits. If you plant this papaya

from the seed then within 6 to 12 months, it will be mature enough to produce the fruit.

Although opinions differ on the origin of *C. papaya* in tropical America (see Garrett 1995), it is likely that *C. papaya* originates from the lowlands of eastern Central America, from Mexico to Panama (Nakasone & Paull 1998). Its seeds were distributed to the Carribean and south-east Asia during Spanish exploration in the 16th Century, from where it spread rapidly to India, the Pacific and Africa (Villegas 1997).

Papaya is now grown in all tropical countries and many sub-tropical regions of the world. It was deliberately introduced to Australia more than a century ago as a horticultural crop for fruit production (Garrett 1995)

2.1.1 Uses of Papaya

Economically, *Carica papaya* is the most important species within the Caricaceae, being cultivated widely for consumption as a fresh fruit and for use in drinks, jams candies and as dried and crystallised fruit (Villegas 1997). Green fruit and the leaves and flowers may also be used as a cooked vegetable (Watson 1997). Nutritionally, papaya is a good source of calcium and an excellent source of vitamins A and C (Nakasone & Paull 1998). The vitamin A and C content of one medium papaya approaches or exceeds USDA minimum daily requirements for adults (see OECD 2003). The fruit of some species of *Vasconcella* may be used as a food source, particularly in some regions of South and Central America, but such usage is relatively limited.

Papaya also has several industrial uses. Biochemically, its leaves and fruit are complex, producing several proteins and alkaloids with important pharmaceutical and industrial applications (El Moussaoui et al. 2001). Of these, however, papain, is a particularly important proteolytic enzyme that is produced in the milky latex of

green, unripe papaya fruits (note that ripe papaya fruit contain no latex or papain). The latex is harvested by scarifying the green skin to induce latex flow, which is allowed to dry before collection for processing (Nakasone & Paull 1998). Evolutionarily, papain may be associated with protection from frugivorous predators and herbivores (El Moussaoui et al. 2001). Commercially, however, papain has varied industrial uses in the beverage, food and pharmaceutical industries including in the production of chewing gums, in chill-proofing beer, tenderising meat, drug preparations for various digestive ailments and the treatment of gangrenous wounds. Papain has also been used in the textiles industry, for degumming silk and for softening wool (Villegas 1997) and in the cosmetics industry, in soaps and shampoo.

2.1.2 Morphology of Papaya

Carica papaya is a soft-wooded perennial plant that lives for about 5-10 years, although commercial plantations are usually replanted sooner (Chay-Prove et al. 2000). Papayas normally grow as single-stemmed trees with a crown of large palmate leaves emerging from the apex of the trunk, but trees may become multi-stemmed when damaged (Villegas 1997). The soft, hollow, cylindrical trunk ranges from 30 cm diameter at the base to about 5 cm diameter at the crown. Under optimal conditions, trees can reach 8-10 metres in height but in cultivation, they are usually destroyed when they reach heights that make harvesting of fruit difficult (Villegas 1997). Cultivated trees in Australia are usually replaced before exceeding 4 m in height.

Papaya flowers are born on inflorescences which appear in the axils of the leaves. Female flowers are held close against the stem as single flowers or in clusters of 2-3 (Chay- Prove et al. 2000). Male flowers are smaller and more numerous and are born on 60- 90 cm long pendulous inflorescences (Nakasone & Paull 1998). Bisexual flowers are intermediate between the two unisexual forms (Nakasone & Paull 1998). The functional gender of flowers can be altered or reversed, depending on environmental conditions, particularly temperature.

Fruit are ready to harvest five to six months after flowering, which occurs five to eight months after seed germination (Chay-Prove et al. 2000). The fruits range in size from 7-30 cm long and vary in mass from about 250 to 3000g (OECD 2003). Fruit from female trees are spherical whereas the shape of fruit from bisexual trees is affected by environmental factors, particularly temperature, that modify floral morphology during early development of the inflorescence (Nakasone & Paull 1998)

2.2 Plants as Sources of Antioxidants

Natural antioxidants may be found in any plant part. Fruits, vegetables, spices, nuts, seeds, leaves, roots and barks have been considered as potential sources of natural antioxidants (Pratt and others 1990). Antioxidants in flaxseed, sunflower, soybean, cottonseed and papaya typify those found in oilseeds. The majority of natural antioxidants are phenolic compounds, and the most important groups of natural antioxidants are the tocopherols, flavonoids and phenolic acids that are common to all plant sources (Naczk and Shahidi 2006).

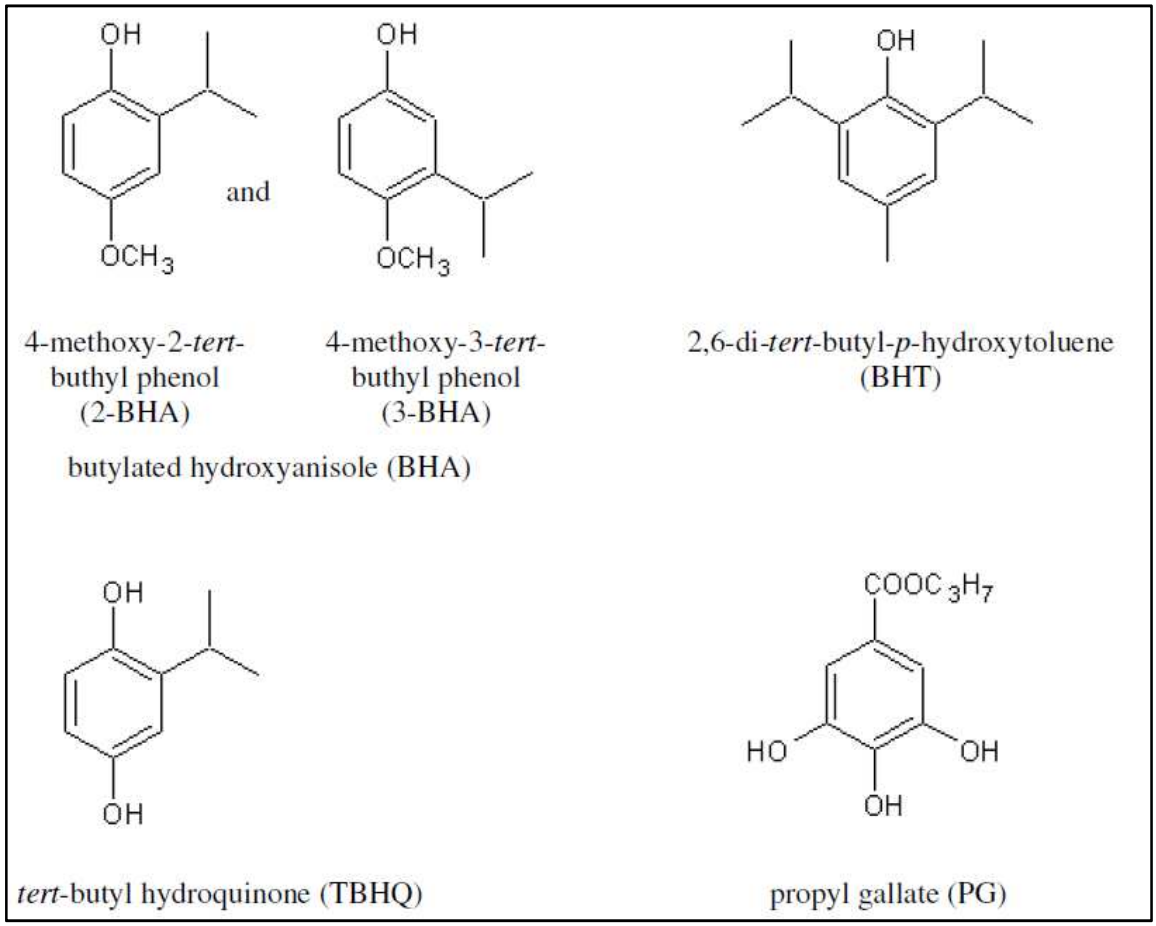

Figure 2.2 Chemical structures of food-grade synthetic phenolic antioxidants (modified from Yanishlieva 2001)

Flavonoids represent a large group of phenolics that occur naturally in plants andare found in fruits, vegetables, grains, barks, roots, stems, flowers, tea and wine (Blokhina and others 2003). They are characterized by the carbon skeleton C6–C3–C6.The basic structure of these compounds consists of two aromatic rings linked by a threecarbonaliphatic chain (Yanishlieva 2001). Several classes of flavonoids are delineatedon the basis of their molecular structure, but the four main groups that occur in planttissues are flavones, flavanones, catechins and anthocyanins (Figure 2.3) (Nijveldt andothers 2001).

Phenolic acids and their derivatives occur widely in the plant kingdom, *e.g.*,legumes, cereals, fruits and plant products such as tea, cider, oil, wine, beverages and medicinal plants (Odaci and others 2007). Phenolic acids (Figure 2.4) can found

in free and conjugated forms in cereals (Naczk and Shahidi 2006). They are present in highest concentration in the aleurone layer of grains, but are also found in the embryo and seed coat (Naczk and Shahidi 2006). The level of phenolics in plant sources also depends on such factors as cultivation techniques, cultivar, growing conditions, ripening process, processing and storage conditions, as well as stress conditions such as UV radiation, infection by pathogens and parasites, wounding, air pollution and exposure to extreme temperatures (Naczk and Shahidi 2006).

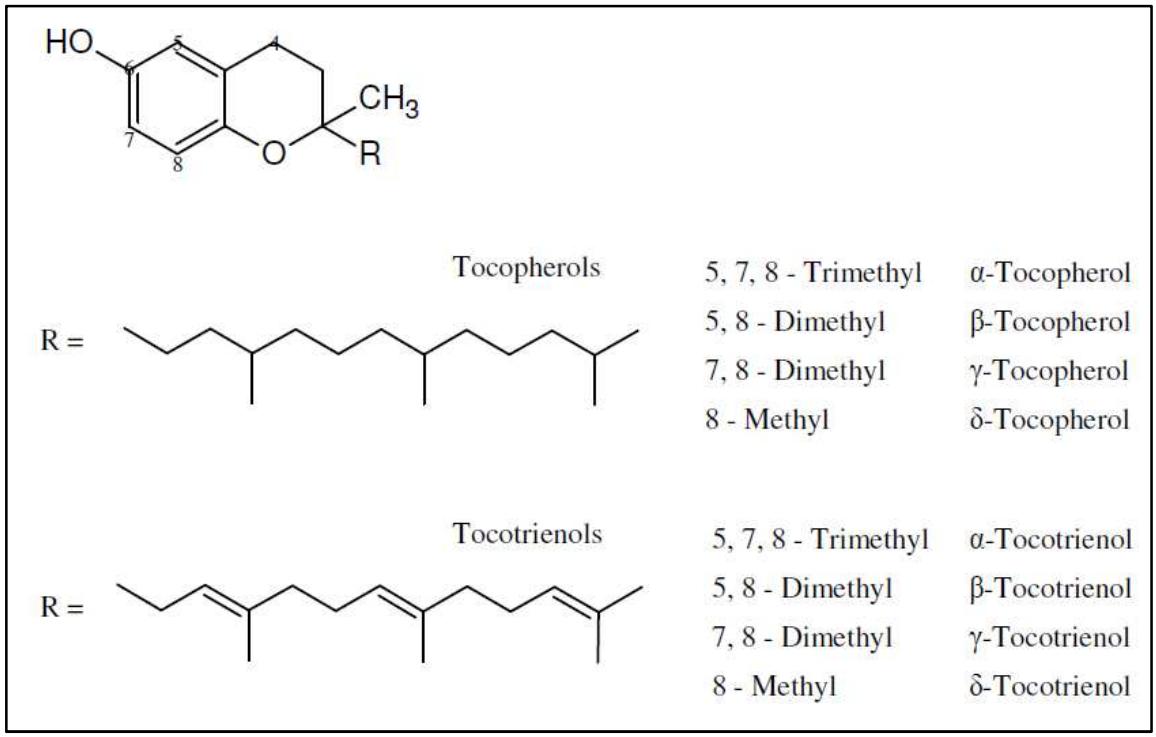

Figure 2.3 Chemical structures of tocopherols and tocotrienols and their isomers (modified from Yanishlieva 2001).

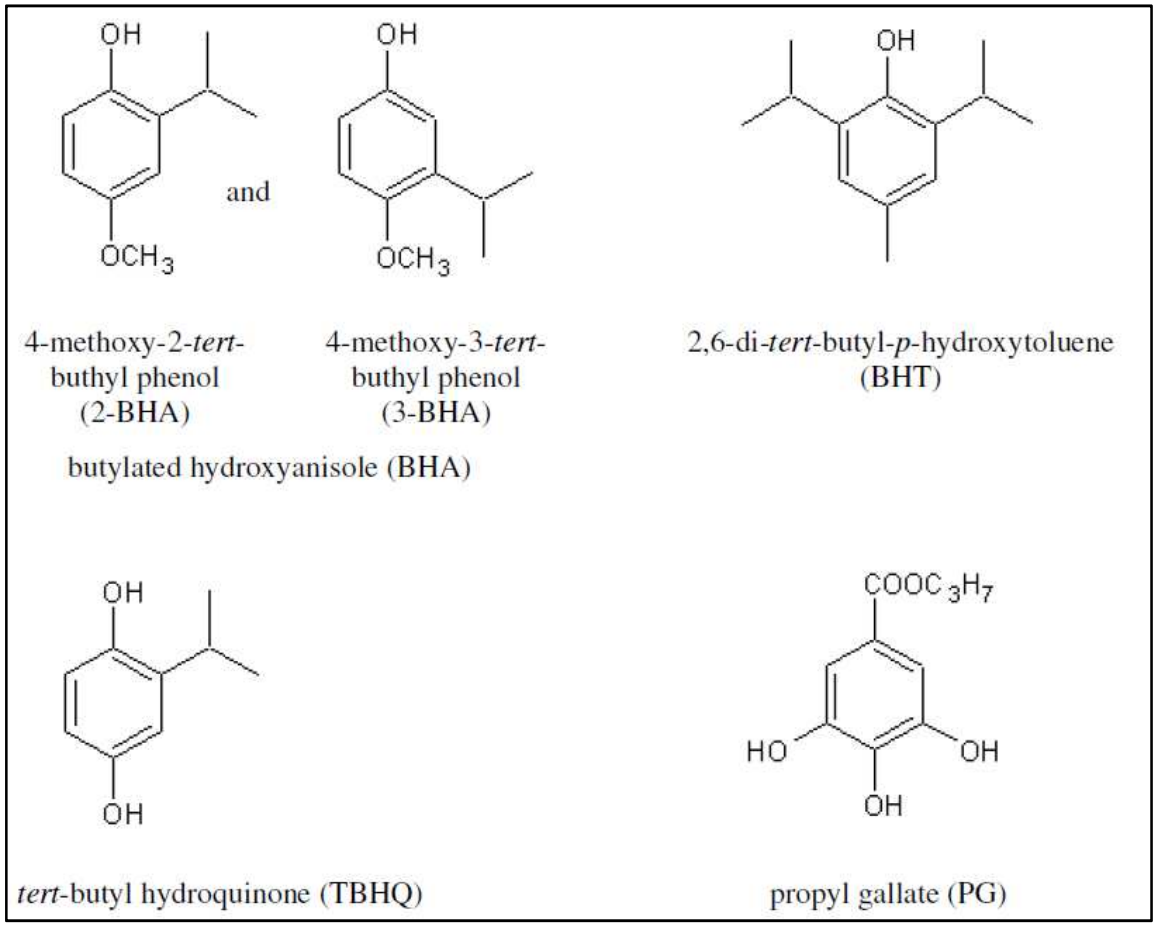

Figure 2.4 The molecular structures of the four main flavonoid groups (modified from Nijveldt and others 2001)

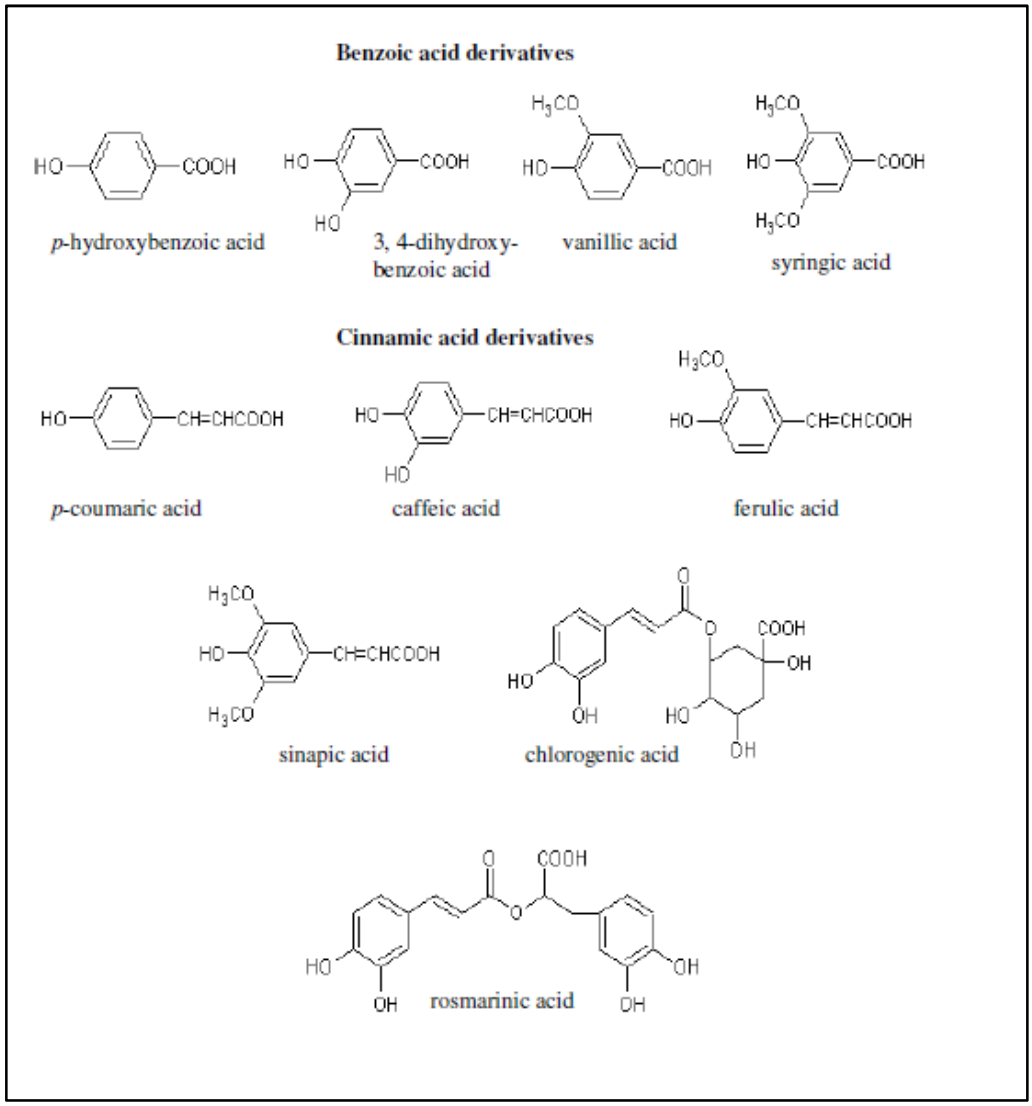

Figure 2.5 Phenolic acids as examples of common natural antioxidants (modified from Yanishlieva 2001)

2.2.1 Antioxidants in Papaya

Papaya is a derivative of rapeseed with low glucosinolate and erucic acid contents (Hall 2001). Antioxidant compounds identified in papaya include phenolic acids (both benzoic and cinnamic acid derivatives) (Kozlowska and others 1988), flavonoids (Hall 2001) and condensed tannins (Shahidi and Naczk 1989). A diversity of phenolic compounds is present in papaya and rapeseed flours (dehulled, defatted seed), meals (defatted, whole seed) or extracts, indicating that these products could

protect a food against rancidity by any of several mechanisms (Table 2.1). The high antioxidant activity of a papaya fraction containing several groups of phenolics demonstrated protection via multiple mechanisms (Amarowicz and others 2003).

Papaya meal has been reported to contain 15.4-18.4 g/kg (dry basis, defatted meal) of phenolic acids (Shahidi and Naczk, 1992). The phenolic compounds of flesh and peel include hydroxylated derivatives of benzoic acid and *trans*-cinnamic acid, coumarins, flavonoids and lignans (Pink and others 1994). Phenolic acids in papaya meal are found in free, esterified or insoluble-bound forms (Naczk and others 1998). These authors reported that papaya meal may contain more than 2 g of free phenolic acids per kg of meal, more than 15 g of esterified phenolic acids per kg of meal and approximately 1 g of insoluble-bound phenolic acids per kg of meal (dry basis in all cases).

A high concentration of sinapic acid in papaya meal was reported by Naczk and others (1992). These authors also reported that sinapic acid, the predominant phenolic acid found in papaya, exists in free form, in esterified form, and in soluble-bound form. Wanasundara and others (1995) reported that sinapic acid and its analogues contributed significantly to antioxidant in papaya meal. Several compounds with high antioxidant were identified as phenolic compounds having one, two or three 14hydroxy groups (Figure 2.5), which were identified (by thin layer chromatography) assinapic acid, *p*-hydroxybenzoic acid, flavonoids and 1-*O*-_-D-glucopyranosyl sinapate (Wanasundara and others 1995).

Wanasundara and Shahidi (1994) reported that the antioxidant of a crude ethanolic extract of papaya meal (500 and 1000 ppm) against the oxidation of papaya flesh was equivalent to that of TBHQ (200 ppm), and stronger than that of BHA (200 ppm),BHT (200 ppm) or BHA/BHT/monoglyceride citrate (MGC) (250 ppm) on a mass basis. Wanasundara and others (1994) isolated the most active component of the extract and identified it as 1-*O*-_-D-glucopyranosyl-3, 5-dimethoxy-4-hydroxycinnamate (1-*O*-_-Dglucopyranosylsinapate; Figure 2.6). Shahidi and others (1995) observed that the addition of 0.5-5% papaya provided 73-97% inhibition of